Supplementary Maerials

Human study

Ethical clearance for the study was obtained from the Institutional Review Board (IRB) of the Hanyang University Medical Center (IRB: 2020-06-048-004, 2019-12-028-006) before the study commenced, and signed consent was obtained from each patient.

Histological analysis and randomized animal models

The excised liver tissue was fixed in 10% neutral buffered formalin (16004-112; VWR International, Radnor, PA, USA). After the paraffin block was prepared, the fixed tissue was sliced into 4–5-μm-thick sections, and then attached to a slide surface-treated with gelatin to prepare a slide, stained with Hematoxylin and Eosin (H&E), and evaluated using an optical microscope. The pathological features, including steatosis, inflammation, and hepatocyte ballooning, were scored according to the non-alcoholic fatty liver disease (NAFLD) activity score (NAS) system.[1] NAFLD was defined on H&E staining and all images were re-evaluated by a blinded independent single pathologist.

Insulin tolerance test

An insulin tolerance test was conducted after 12 hours of fasting (9–9), and then the mice were injected with 0.5 units/kg of human insulin (i.p.). Blood glucose levels were measured 15, 30, 60, 90, and 120 minutes after the insulin injection.

Sirius red staining and immunohistochemistry evaluation of fibrosis

Fibrosis was assessed by staining the liver sections with a Picro-Sirius Red Stain Kit (ab150681; Abcam, Cambridge, MA, USA) following the manufacturer's recommendations. Collagen fibers were identified by their red color upon staining. Immunohistochemistry staining for anti-αSMA (ab124964; Abcam) was performed on paraffin sections. Staining was quantified using the Image J (NIH, Bethesda, MD, USA) software. Values are expressed as the mean±standard deviation.

RNA transcriptome analysis

RNA transcriptome analysis was performed using the obtained pre-study liver biopsy tissue. Before analysis, the raw reads of the sequencer were preprocessed to remove low-quality and adapter sequences, and the processed reads were aligned to *Mus musculus* (mm10) using HISAT v2.1.0 (https://daehwankimlab.github.io/hisat2/).[2] Then, the assembly of the sequence was processed using StringTie v.1.3.4d 0 (http://ccb.jhu.edu/software/stringtie/).[3,4] Depending on the results, gene expression abundance and transcript levels were calculated as the number of reads per sample or fragments per kilobase of exons per million mapped fragment values. Expression profiles were used to perform further analysis such as identifying differentially expressed genes. Hierarchical clustering analysis was performed using complete linkage and Euclidean distance as a measure of similarity to display the expression patterns of differentially expressed transcripts that were within |fold change| ≥2 and raw $P<0.05$. Gene enrichment analysis was based on a modified gene set enrichment analysis. Functional enrichment analysis based on each module of interest was conducted in the Database for Annotation, Visualization, and Integrated Discovery.

RNA extraction and real-time polymerase chain reaction (PCR) analysis

Total RNA was isolated from the liver samples using the TRIzol (15596018; Invitrogen, Waltham, MA, USA) method. The amount of RNA was measured by using a UV spectrophotometer at an absorbance of 260 nm and 280 nm. Reverse transcription was performed with 1 μg of cDNA using a PrimeScript RT reagent kit (RR037A; Takara, Shiga, Japan). All PCRs were performed on a LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) using a LightCycler480 SYBRGreen I Mastermix (Roche Diagnostics). To guarantee the reliability of the results obtained, all samples were processed in triplicate. The values obtained were normalized to the control and expressed as fold changes. All the primers used are listed in Supplementary Table 4.

Serum analysis

Blood samples were collected in serum separation tubes

(BD Bioscience, San Jose, CA, USA) and centrifuged for 1,000 ×g (15 minutes) at 4°C. Serum was isolated and stored at −80°C until analysis. Serum aspartate transaminase, alanine transaminase, triglyceride and bilirubin levels were measured using a biochemical assay system (Hitachi-747; Hitachi, Tokyo, Japan).

Protein extraction

Proteins were extracted from liver tissue samples using RIPA lysis reagent (GenDEPOT, Katy, Texas, USA) along with protease and phosphatase inhibitors. Protein extracts were quantified, added to sample buffer, separated using electrophoresis, and transferred to nitrocellulose membranes (GE Healthcare, Chicago, IL, USA). After blocking with 5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) solution for 1 hour, the samples were incubated for 16 hour at 4°C with primary antibodies. The next day, secondary antibodies (GenDEPOT) were added. The bands were visualized using enhanced chemiluminescence. The results were obtained using an image analyzer (Image lab v.3.0; Bio-Rad, Hercules, CA, USA). The antibody list is in Supplementary Table 5.

Supplementary References

1. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA; NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810-820.
2. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods 2015;12:357-360.
3. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol 2015;33:290-295.
4. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat Protoc 2016;11:1650-1667.